

Long-Term Hypothyroidism and Hypothalamus-Pituitary-Adrenal (HPA) Response in Adult Mice¹

LEE A. MESERVE AND EDMOND V. RUSS, III, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403-0212

ABSTRACT. Previous studies of the hypothalamus-pituitary-adrenal (HPA) axis in young rodents have found thiouracil-induced hypothyroidism to delay development of the axis past a relative stress non-responsive period. Little attention has been paid to the effects of long-term hypothyroidism on the function of this axis. The present study examined HPA axis response to an acute stress as determined by production of the glucocorticoid corticosterone, in mice made hypothyroid by thouracil exposure from conception to adulthood. The effect of injecting thyroxine (T_4) or triiodothyronine (T_3) on HPA response in these animals was also determined. Long-term hypothyroidism resulted in depressed body weight, subnormal levels of circulating T_4 , and elevated levels of T_3 . However, HPA axis response to an acute stress was normal in hypothyroid mice, and was not augmented by T_4 injection for two weeks. On the other hand, two weeks of T_3 injection allowed for a 70% increase in stress response as compared to either euthyroid or hypothyroid animals. The basis of the differential effect of the two thyroid hormones on stress response in hypothyroid mice remains to be determined.

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INTRODUCTION

Early in the study of mammalian physiological response to stress, the hypothalamus-pituitary-adrenal (HPA) axis was described (Selye 1959), and the control of glucocorticoid secretion by this axis was recognized as adaptively advantageous. Subsequent study of ontogeny of the HPA axis in experimental rodents revealed a refractory period during the first two weeks of life that was called the stress non-responsive period (Shapiro et al. 1962). Recent investigators have found the non-responsiveness to stress to be relative, rather than absolute as was originally thought, depending upon the axis parameter being measured (Walker et al. 1991). Generation of a pronounced response by this axis requires maturation of hypothalamic production and secretion of corticotropin-releasing factor (CRF), of pituitary production and secretion of adrenocorticotrophic hormone (ACTH), and of adrenal production and secretion of glucocorticoid. Work in our laboratory has found that hypothyroidism induced perinatally by thiouracil in rats (Meserve and Leathem 1981) and mice (Meserve 1976), by genetic mutation (*hyt*) in mice (Meserve 1987), and more recently by polychlorinated biphenyl (PCB) in rats (Meserve et al. 1992) delays developmental progression past the relative stress non-responsive period when elevation of circulating glucocorticoid (corticosterone in rats and mice) is taken to indicate HPA response. Fewer data have been collected regarding the influence of prolonged hypothyroidism on the response of the HPA axis to a stressful stimulus. Additionally, the influence, after long-term deficiency, of exogenous thyroid hormone upon HPA function has not been determined. The present study was designed to investigate HPA axis function in mice exposed to dietary thiouracil from conception to adulthood, and to determine whether

injection of exogenous thyroid hormone modified this function.

MATERIALS AND METHODS

Adult female Swiss-Webster mice were obtained from the Animal Research Facility at Bowling Green State University, and were mated to males of the same strain. From the day of conception as determined by vaginal plug, pregnant females were housed singly and fed Wayne Lab Blox Mash (Continental Grain Co., Chicago, IL) with or without 2-thiouracil (0.25%, w/w; Sigma Chemical Co., St. Louis, MO) to induce hypothyroidism. Litters were reduced to eight pups at five days of age. Animals were removed from the maternal cage at 25 days of age and were housed in pairs, where they continued to receive the same diet provided to the mother. Animals were between six and eight months of age when assigned to treatment groups. Male and female animals were randomly assigned to treatment groups, since no gender differences were revealed in the parameters tested. A total of 50 mice, 10 per group, were assigned to the following treatment groups:

1. Control - Fed no thiouracil, given no thyroid hormone injections (CON).
2. Thiouracil, uninjected - fed thiouracil-containing diet, given no thyroid hormone injections (THIO-NI).
3. Thiouracil, saline-injected - fed thiouracil-containing diet, injected with physiological saline (0.9% NaCl) vehicle (0.1 ml, sc) (THIO-SAL).
4. Thiouracil, triiodothyronine (T_3)-injected - fed thiouracil-containing diet, injected with T_3 (25 ng/g b wt, sc, in 0.1 ml saline) (THIO- T_3).
5. Thiouracil, thyroxine (T_4)-injected - fed thiouracil-containing diet, injected with T_4 (50 ng/g b wt, sc, in 0.1 ml saline) (THIO- T_4).

Animals receiving either injection vehicle or thyroid

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hormone were injected with the indicated dose once daily for 14 days.

To determine HPA axis response, each group of mice was divided into non-stressed and stressed subgroups. Non-stressed animals (five mice per group) were decapitated immediately after removal from the home cage, and stressed animals (five mice per group) were decapitated 15 min after a 1 min exposure to ether fumes. To avoid possible circadian effects on corticosterone concentration, decapitation was always performed between 0800 and 0900 (lights on at 0700; lights off at 1900). Blood was collected at decapitation and allowed to clot at room temperature, and serum was removed by centrifugation for determination of thyroid hormone and corticosterone content. Serum was stored frozen at -20°C . Body weight was determined to the nearest 0.1 g, and liver and thyroid gland weights were determined to the nearest 0.1 mg. Analysis of serum concentrations of T_4 , T_3 , and corticosterone was done by radioimmunoassay, using commercially available kits (ICN Biomedicals, Carson, CA). For a given hormone, all samples were assayed in one run. The interassay variation was $<5\%$ in each case. Data were statistically analyzed using one-way analysis of variance, with significance ascribed where $p < 0.05$. In cases of significance, multiple comparisons of means were made using the Student-Newman-Keuls test (Zar 1984).

RESULTS

Long-term thiouracil feeding depressed average body weight to 74% of normal, and two weeks of thyroid hormone administration did not alter this depression (Fig. 1A). Thyroid glands displayed the expected goitrogenesis in response to thiouracil, with the mass of the gland relative to body weight increasing to greater than five times control (Fig. 1B). Two weeks of T_4 replacement significantly decreased this goitrogenesis, but did not eliminate it completely, while T_3 injections were without effect. Neither thiouracil alone, nor administration of thyroid hormone with thiouracil, significantly altered liver weight (data not shown).

Circulating T_4 levels were depressed to 15% of normal by thiouracil feeding (Fig. 2A), and neither saline injection vehicle nor T_3 altered this depression. Two weeks of T_4 replacement increased circulating levels to nearly four times those of euthyroid controls. Conversely, thiouracil feeding elevated circulating T_3 to 168% of normal. Saline and T_4 did not influence this elevation, but T_3 injection normalized circulating T_3 to a value not significantly different from controls (Fig. 2B).

Thiouracil did not significantly modify non-stressed levels of corticosterone (Fig. 3). The handling required for the injection process resulted in a lesser mean corticosterone value in non-stressed thiouracil-fed animals that were injected with saline for two weeks. Thiouracil did not suppress the elevation of circulating corticosterone in response to a general stressor. Administration of T_4 to hypothyroid mice did not alter non-stressed or stressed corticosterone concentration, but T_3 augmented the stress response of hypothyroid animals by greater than 70%.

DISCUSSION

Long-term feeding of thiouracil to mice in the present study resulted in the characteristics of hypothyroidism previously reported when thiouracil was administered to rats (Blake and Henning 1985). Specifically, body weight was depressed, the thyroid gland became goitrous, and circulating thyroxine concentration was markedly subnormal (Figs. 1, 2). Since transcription of the growth hormone gene is stimulated by thyroid hormone (Cassanova et al. 1985), and since recent investigations have found compensatory down-regulation of receptors for hypothalamic growth hormone regulatory peptides in hypothyroid rats (Tan et al. 1996), it is not surprising that the thiouracil-fed mice in the present study were smaller than controls. Although human studies have found T_4 replacement to institute catch-up growth in children who have been chronically hypothyroid (Boersma et al. 1996), two weeks of thyroid hormone therapy did not improve body weight of mice in the present study (Fig. 1). It would be of interest to determine whether thyroid replacement for a longer time would restore body weight in similar mice.

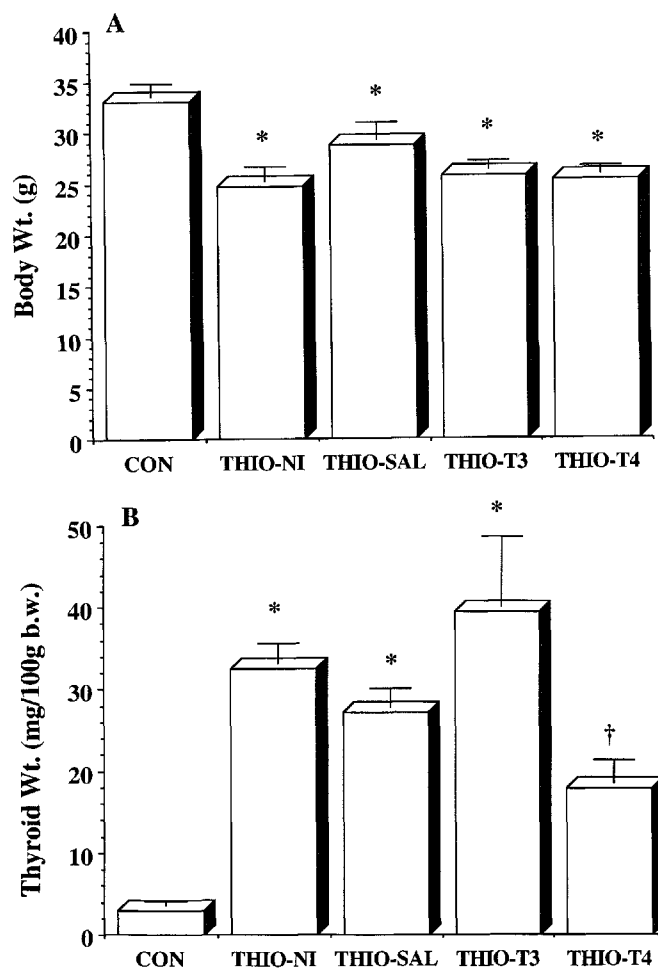


FIGURE 1. Effect of thiouracil feeding and thyroid hormone injection on body weight (panel A) and thyroid gland weight relative to body weight (panel B). Each bar represents mean \pm SEM for 10 mice. CON - control; THIO-NI - thiouracil-fed, uninjected; THIO-SAL - thiouracil-fed, saline-injected; THIO-T₃ - thiouracil-fed, T₃-injected; THIO-T₄ - thiouracil-fed, T₄-injected. *Significantly different from controls ($p < 0.05$). †Significantly different from every other group ($p < 0.05$).

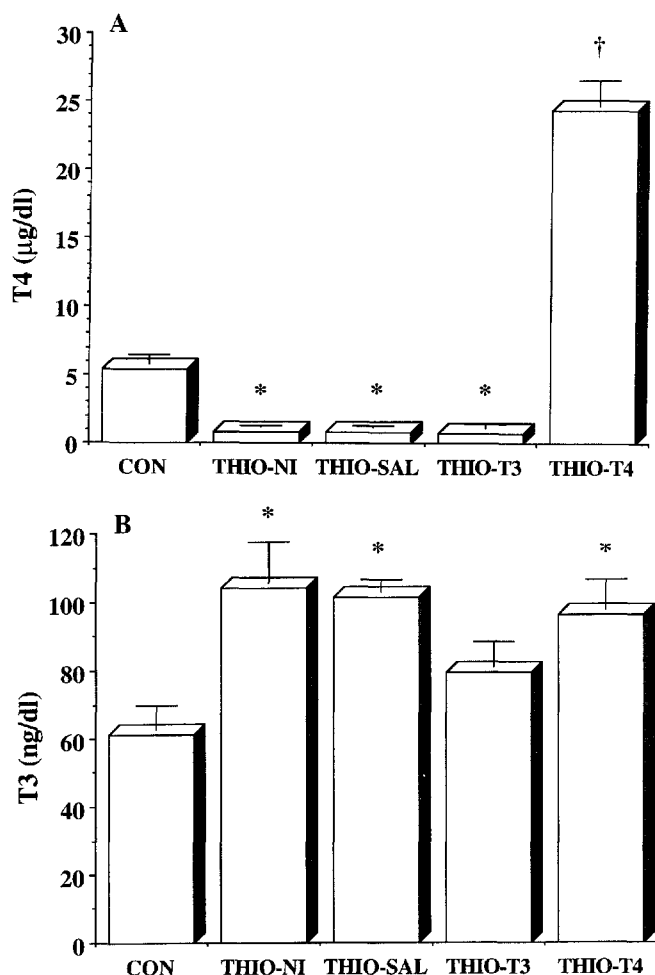


FIGURE 2. Effect of thiouracil feeding and thyroid hormone injection on circulating thyroxine (T_4 ; panel A) and triiodothyronine (T_3 ; panel B) concentrations. Each bar represents mean \pm SEM for 10 mice. Bar labels are the same as in Fig. 1. *Significantly different from controls ($p < 0.05$). †Significantly different from every other group ($p < 0.05$).

Despite displaying some characteristics representative of hypothyroidism, stress response of the HPA axis to a general stressor was not subnormal at this age (Fig. 3). Indeed, the depression of non-stressed corticosterone levels in thiouracil-fed animals sham-injected with saline for two weeks (Fig. 3, THIO-SAL) reflected the sensitivity of the HPA axis to handling as has been previously reported in euthyroid animals (Meaney et al. 1988). Previous studies of individual HPA axis components in young (15 day) hypothyroid rats have found hypothalamic CRF content to be subnormal, while pituitary ACTH content and adrenal response to ACTH remain normal (Meserve and Pearlmuter 1983), suggesting a primary defect in the axis at the level of the hypothalamus.

Given that the adult mice in the present study produced a normal HPA response regardless of thyroid status, it appears that the hypothalamic component of the axis can mature sufficiently to produce, store, and release CRF despite thiouracil feeding. This is likely the result of a retarded progression of developmental processes allowed by small quantities of circulating T_4 in the hypothyroid animals, rather than by elevated T_3 , since neurons and glia rely almost exclusively on T_4 as a

thyroid hormone source (Garcia-Segura et al. 1996), which is then converted to T_3 intracellularly (McNabb 1995). This neural preference for T_4 results, at least in part, from delivery of T_4 to the brain by the protein transthyretin (Schreiber et al. 1995).

Under normal circumstances, about 80% of the T_3 in circulation is produced by peripheral deiodination of T_4 , primarily accomplished in the liver and the kidneys (McNabb 1992). However, the action of the Type I 5'-deiodinase enzyme responsible for this conversion is inhibited by thiouracil (Oppenheimer et al. 1972). Thus, one might anticipate a depression of T_3 in thiouracil-fed mice, rather than an elevation as seen in the present study (Fig. 2). That T_3 is elevated may result from a number of compensatory mechanisms, including the following. 1) Under circumstances where production of T_4 by the thyroid is depressed either spontaneously or experimentally, the fraction of thyronine iodinated with fewer than four atoms of iodine (including T_3) increases (McNabb 1992). The prolonged suppression of thyroxine production by thiouracil feeding may lead to the elevated T_3 levels displayed in the current study. 2) Antithyroid substances, such as thiouracil, increase the activity of liver enzymes that glucuronidate thyroid hormones, bringing about their excretion in liver bile. The activity of the species of glucuronidase that uses T_3 as a substrate is less markedly elevated by thiouracil than is that of the one which favors T_4 (Visser et al. 1996). The preference for T_4 glucuronidation might result in elevation of circulating T_3 over time. 3) Thiouracil has long been known to modify protein metabolism in the liver (Yatvin et al. 1964). Given that transthyretin is produced by the liver, thiouracil may depress circulating levels of this exclusive T_4 transport protein, resulting in a larger fraction of T_3 in circulation, since T_3 binds to more abundant plasma proteins. It is unlikely that any of these explanations individually accounts for the paradoxical elevation of T_3 in thiouracil fed animals. There is undoubtedly some influence of all of these alterations, and others yet to be identified.

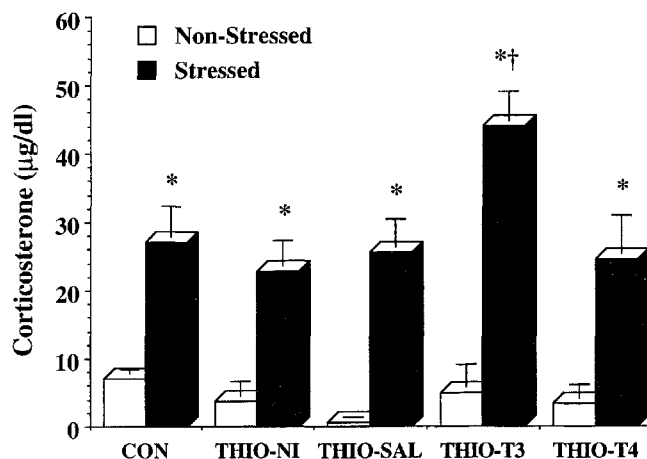


FIGURE 3. Effect of thiouracil feeding and thyroid hormone injection on circulating corticosterone concentration in non-stressed and stressed mice. Each bar represents mean \pm SEM for five mice. Bar labels are the same as in Fig. 1. *Significantly different from non-stressed animals ($p < 0.05$). †Significantly different from every other group ($p < 0.05$).

Since the HPA response to stress was normal in untreated (THIO-NI) and sham-treated (THIO-SAL) mice fed thiouracil in the present study (Fig. 3), one might anticipate that thyroid hormone injection would not augment this response. Indeed, this was the case with T_4 injection, but not with T_3 administration. The question then arises, why did provision of a hormone already circulating in excess (T_3) result in an enhanced response, while injection of one obviously deficient in circulation (T_4) did not? Although they remain speculative, any one or a combination of the following factors may have brought about this result. 1) It is possible that a sizable fraction of the elevated T_3 in thiouracil-fed mice was bound to plasma albumin (McNabb 1992), and thus inaccessible to target cells. Injection of T_3 would provide, on a daily basis, an increase in the free fraction of this hormone to stimulate target cells, such as those in the adrenal cortex responsible for corticosterone production and secretion. 2) Although T_4 injection elevated circulating levels of the hormone in thiouracil-fed mice, the HPA axis sites sensitive to this hormone (hypothalamus and pituitary) may have been maximally activated by the previous small amounts of T_4 in circulation, or by other iodothyronines (e.g., T_2 has recently been suggested as a physiologically important molecule; Cimmino et al. 1996). Additionally, activation of free T_4 -clearing mechanisms such as glucuronidation (Visser et al. 1996) may be sufficient to minimize the influence of elevated total T_4 in thiouracil-fed mice injected with T_4 . 3) Given that hypothalamic and pituitary components of the HPA axis rely primarily on T_4 as their circulating thyroid hormone, perhaps T_3 had a primary stimulatory effect on the cells of the adrenal cortex (as noted in 1 above), increasing the efficiency of adrenal response to ACTH with augmented glucocorticoid release. This suggestion of variable adrenal responsiveness has been supported by evidence in five rat strains with differing HPA response to the same stress, that the difference is primarily at the adrenal, rather than the hypothalamus or pituitary (Gómez et al. 1996).

In summary, adult mice were able to generate a normal HPA response to acute stress, despite severely depressed circulating T_4 resulting from long-term thiouracil feeding. Augmentation of thyroid status by T_4 injection did not alter HPA response, but injected T_3 allowed a 70% enhancement of corticosterone secretion in response to stress. The basis of this differential effect of injected thyroid hormones remains to be determined.

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